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VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture*

ABSTRACT

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Spore populations of vesicular-arbuscular mycorrhizal (VAM) fungi and formation of mycorrhizae in maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) were studied in three farming systems: a conventional maize–soybean rotation and two low-input systems. Spore populations were counted in soil samples obtained at planting and after harvest for two growing seasons. Maize and soybean root systems were sampled for mycorrhizae early in the growing season. Low-input plots tended to have higher populations of spores of VAM fungi than the conventionally farmed plots. Further, the readily identifiable species *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe, was more numerous in low-input plots (up to 30 spores 50 cm^{-3} soil) than in conventional plots (0–0.3 spores 50 cm^{-3} soil), suggesting farming system affected species distribution as well. Colonization of plants in the field did not always reflect VAM fungus spore populations at planting. Greenhouse bioassays showed 2.5–10 fold greater colonization of plants growing in soil from low-input than conventional systems. The results indicate that conventional farming systems yield lower levels of VAM fungi whereas low-input sustainable agriculture, with cover crops planted between cash crops, has greater populations of VAM fungi and potential to utilize the benefits of VA mycorrhizae.

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi and plant roots have lived in symbiosis since vascular plants first appeared on land (Stubblefield et al., 1987). They have been shown to impart a variety of benefits upon their host,

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including increased growth and yield (Mosse, 1973). Vesicular-arbuscular mycorrhizal fungi have been shown to enhance the growth of a number of field crops, among them soybean and maize (Ross and Harper, 1970; Khan, 1972). Greenhouse experiments have shown that the growth increase is typically because of increased P uptake (Mosse, 1973).

Environmental and economic considerations are causing agronomists to consider low-input sustainable agriculture (LISA) as an alternative to conventional agriculture. The demonstrated benefits of VAM fungi indicate that these organisms should be crucial for plant growth and economically viable yields under an agricultural practice shunning chemical pest control and fertilizers. A first step in utilizing VAM fungi in LISA is to study the effects of various farming systems upon VAM fungi in order to learn how to manage for these fungi while optimizing grain or forage yield.

Vesicular-arbuscular mycorrhizal fungi are obligate symbionts and as such require fixed carbon from host plants. Long-term fallowing of fields has been shown to be detrimental to VAM fungus populations and results in decreased growth of crops (Thompson, 1987). Tillage and greenhouse experimental treatments designed to simulate tillage decrease the effectiveness of mycorrhizae (Jasper et al., 1989; Evans and Miller, 1990; Fairchild and Miller, 1990), and affected the number (Kruckelmann, 1975) and distribution of VAM fungus spores in the soil (Zhai et al., 1990).

Crop rotations also affect VAM fungi. Monoculture affects both populations of VAM fungus spores (Kruckelmann, 1975) and colonization of roots (Strzemska, 1975). Mycorrhizal and non-mycorrhizal plants in a crop rotation have been shown to increase and decrease, respectively, the populations of VAM fungus spores (Harinikumar and Bagyaraj, 1988; Baltruschat and Dehne, 1988). Pre-cropping savanna land with VAM fungus host plants increased soil inoculum of VAM fungi and increased crop yields the following year when compared with savanna converted directly to crops (Dodd et al., 1990).

Greenhouse experiments studying the effects of fertilizer additions on VA mycorrhizae have shown that application of P decreases both colonization of roots and production of soil-borne spores by VAM fungi (Douds and Schenck, 1990). Application of nitrate, or nutrient solutions without P, usually enhances colonization of roots (Hepper, 1983) and sporulation (Douds and Schenck, 1990).

A field experiment comparing a conventional maize–soybean rotation with two economically and biologically viable LISA systems was initiated with the 1981 growing season (Liebhart et al., 1989). Soybean yields in the LISA systems were equal to or greater than those of the conventional system. Yields of maize in the LISA systems were less than those of the conventional during the first 4 years of the conversion from conventional to LISA, but were equivalent thereafter. Sampling for mycorrhizal fungi to quantify the long-term effect of farming systems upon populations of VAM fungus spores in the soil and formation of mycorrhizae began in 1989.

MATERIALS AND METHODS

Mycorrhizae were examined in an experiment which studied the conversion of farmland from conventional to sustainable agriculture (Liebhart et al., 1989). The field had been utilized for a conventional maize–wheat rotation prior to 1981. Three farming systems, each with a characteristic crop rotation, were initiated in 1981 (Fig. 1). The low-input with animals (LIA) system consisted of a 5-year rotation which utilized green and animal manures and produced some crops for animal feed. The low-input cash grain (LICG) system utilized legume and other cover crops for N and weed control. No chemical N or P fertilizers or pesticides were applied to the LIA and LICG systems. A single application of K_2SO_4 (336 kg ha^{-1}) was made in 1989 in all systems. The final system was a conventional cash grain (CONV) maize (*Zea mays* L.)–soybean (*Glycine max* (L.) Merr.) rotation to which fertilizers and pesticides were applied to Pennsylvania State University recommendations.

Sampling for VAM fungus spores in rhizosphere soil was conducted from autumn 1989 to autumn 1991. Soil samples were collected in the spring, after plowing, and in the autumn after harvest yet before plowing in plots in which maize or soybeans were or would be grown. Spring and autumn samples were necessary to account for rearrangement of soil from moldboard plowing. The field was divided into two blocks, each with two plots. Comly silt loam (fine-loamy, mixed, mesic Typic Fragiudalf) was the dominant soil type. Two rotation points of each farming system appeared within each plot in 9.5m × 95m subplots. Ten soil samples were collected from each rotation point-subplot, evenly spaced along the planting row, two rows from the edge. This yielded 40 samples per farming system rotation point (2 blocks × 2 plots per block × 10 samples per subplot). Soil throughout the top 25 cm of the plow layer was taken from where the crop host was planted. Spores were isolated from 50 cm^3 subsamples via wet sieving (Gerdemann and Nicolson, 1963) and centrifugation (Jenkins, 1964), and identified to genus under a dissecting microscope. Spores so collected and counted were used later to initiate pot cultures in the greenhouse.

Similarly, ten maize and five soybean root systems per block × plot × subplot rotation point were sampled for VAM fungus colonization. Entire root systems were unearthed from evenly spaced intervals along the second row from the plot edge. Fine roots were cleared and stained (Phillips and Hayman, 1970). Soybean roots required additional treatment with alkaline 1.5% H_2O_2. Roots were assayed for VAM fungi using the gridline intersect method (Newman, 1966).

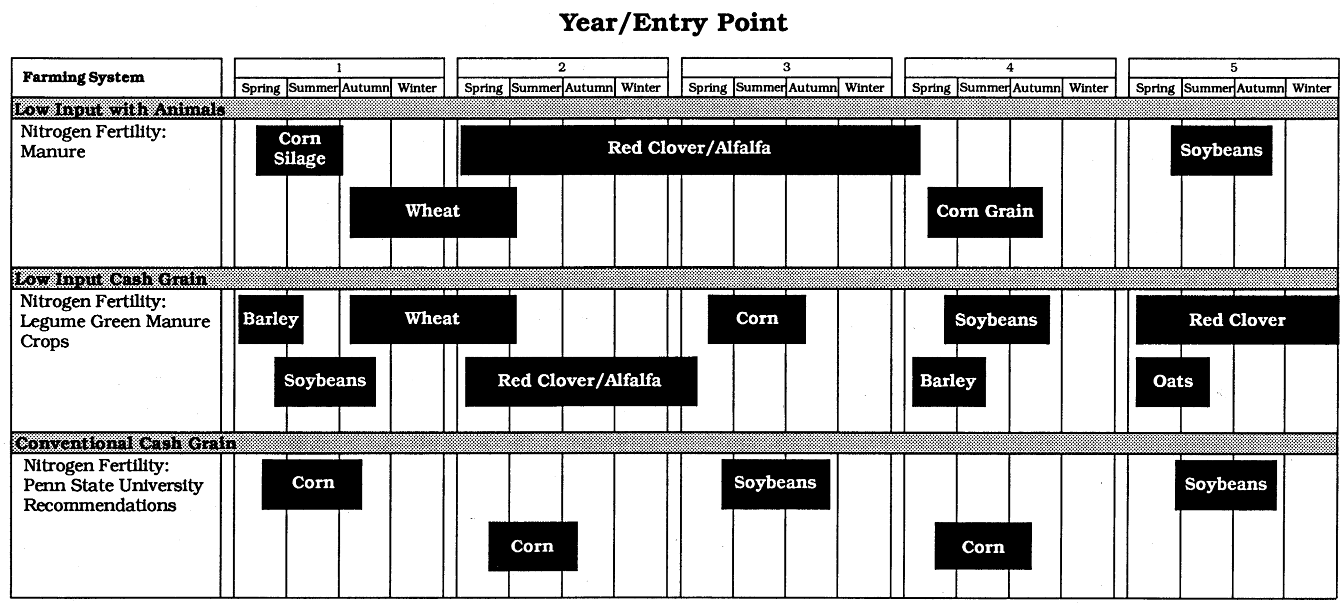

Fig. 1. Farming systems studied.

Greenhouse experiments contrasted the infectivities of soil from the different farming systems. Maize or bahiagrass (*Paspalum notatum* Flugge) were grown in 15 cm diameter plastic pots or plastic cones (Super Cell C-10, Stuewe & Sons, Corvallis, OR), respectively, in 1:1 [v/v] mixtures of soil and vermiculite. Colonization of roots by VAM fungi was measured as above after 3–4 weeks of growth by sampling maize roots within a 50 cm^3 core or entire bahiagrass root systems.

Data were analysed using analysis of variance. Colonization and spore count data were transformed using arcsin and SQRT (X+1), respectively. Measurements for which significant treatment effects were found were characterized further using Tukey's Method of Multiple Comparisons.

RESULTS

Agronomic Data

Soil analyses showed that the field still exhibited P levels reflective of its conventional agriculture history (Table 1). Phosphorus levels were high throughout and soil pH was lower in the LIA and LICG plots. The availability of host plants was much greater in the LISA systems than in the CONV system. Plots under the LISA systems studied here were covered with live plants 70% longer in an average year than plots in the CONV rotation (Fig. 2).

VAM Fungus Spore Populations

Spores isolated from soil samples were placed in three categories: *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe, *Glomus occultum* Walker and *Glomus occultum* - like spores, and other *Glomus* sp. Spores from

TABLE 1

Results of soil analyses, autumn 1989[1]

Farming system	pH[2]	P[3] ($mg\ kg^{-1}$)	Exch. K (meq per 100 g)	Organic[4] matter (%)
Low-input with animals	6.37 b	185 a	0.30 a	3.07 ab
Low-input cash grain	6.36 b	164 b	0.23 b	3.25 a
Conventional	6.83 a	168 b	0.22 b	2.91 b

[1]Numbers in the same column followed by the same letter are not significantly different (Duncan's method of mean separation).
[2]In water.
[3]Bray I extraction (0.025 N HCl and 0.03 N NH_4F).
[4]Walkley–Black method for organic carbon.

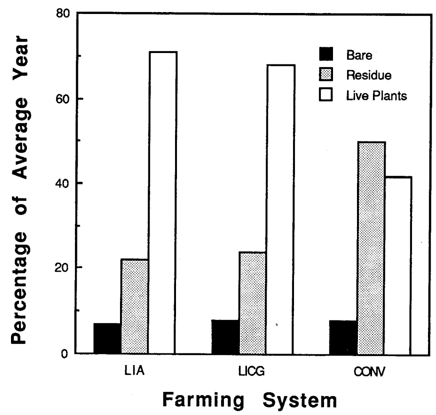

Fig. 2. Percentage of an average year the soil lies bare or is covered by living plants or plant residues for each of the farming systems: LIA, low-input with animals; LICG, low-input cash grain; CONV, conventional cash grain. Data are from 1 April 1981 to 1 April 1990 and assume the soil surface is bare for 1 month following moldboard plowing.

the Gigasporaceae other than *Gigaspora gigantea* were quite rare and are not reported. No spores from field soil could be readily identified as *Acaulospora* or *Entrophospora*, nor were any found in greenhouse pot cultures initiated with spores collected during the assays.

There were qualitative and quantitative differences in populations of VAM fungus spores in soil under conventional vs. LISA management (Table 2). *Gigaspora gigantea* was very uncommon under conventional management, typically just one spore per ten 50 cm^3 samples, yet was quite common in the LICG system, up to 30 spores 50 cm^{-3}. The *Glomus occultum* and *Glomus* sp. groups tended to be more numerous in the LISA systems (overall means of 81.7 and 11.7 spores 50 cm^{-3}, respectively) than in the CONV (overall means of 23.1 and 5.8 spores 50 cm^{-3}, respectively).

Formation of Mycorrhizae

Colonization of maize and soybean in the field by VAM fungi was not completely reflective of spore populations (Table 3). Only data for soybeans in 1990 reflected spore populations at planting. Maize plants in the LICG treatment were sampled 8–18 days later than the other treatments because they were planted 10–12 days later, further complicating sampling for mycorrhizae. Greenhouse experiments were conducted with field soil to combat this problem.

Maize was grown for 4 weeks in the greenhouse with a 1:1 (v/v) mixture of vermiculite and soil from plots planted to maize in 1989 and collected 20 April, 1990. Roots of plants in soil from the LISA systems had greater mycor-

TABLE 2

Populations of VAM fungus spores in the soil at the Rodale Research Center[1]

Farming system	Spores 50 cm^{-3} soil														
	October 1989			April 1990			October 1990			May 1991			October 1991		
	Glomus	GGGT[2]	LOCT[3]	*Glomus*	GGGT	LOCT	*Glomus*	GGGT	LOCT	*Glomus*	GGGT	LOCT	*Glomus*	GGGT	LOCT
Maize (1989)–Soybeans (1990)–Maize (1991)															
Low input with animals		–[4]		8.0 b	2.6 b	128.9 b	4.7 b	0.0 a	48.0 b	7.4 a	0.0 a	66.9 a	20.8 a	5.3 b	109.4 b
Low input, cash grain	11.2 a	5.6 a	47.4 a	13.0 a	6.1 a	243.4 a	18.9 a	0.5 a	94.7 a	11.8 a	1.8 a	83.2 a	21.7 a	25.7 a	139.8 a
Conventional	7.0 b	0.3 b	27.7 b	3.7 c	0.2 c	39.3 c	3.8 b	0.1 a	26.4 c	3.5 a	0.1 a	21.8 b	8.1 b	0.3 c	27.3 c
Soybeans (1989)–Maize (1990)–Soybeans (1991)															
Low input with animals	6.7 a	0.7 a	0.1 a	4.9 a	1.1 a	32.3 a	9.5 b	0.4 b	48.2 b	14.5 a	2.0 b	71.3 a		–[4]	
Low input, cash grain	10.3 a	1.0 a	1.9a		–[4]		17.2 a	30.3a	141.7 a	8.9 a	7.8 a	74.8 a	9.7 a	3.2 a	56.4 a
Conventional	9.8 a	0.0 b	3.4 a	4.5 a	0.1 b	20.2 a	5.1 b	0.1 c	27.3 c	7.9 a	0.1 c	20.4 b	5.0 b	0.0 b	16.9 a

[1]Each number represents the mean of 40 observations. Numbers within a column, within a maize–soybean sequence, followed by the same letter are not significantly different ($\alpha=0.05$, Tukey's Method of Multiple Comparisons). Spore counts were analyzed with ANOVA after SQRT(X+1) transformation.
[2]*Gigaspora gigantea.*
[3]*Glomus occultum* and *G. occultum*-like spores.
[4]Soil samples were not collected from these subplots at this sample period because small grain or other cover crops were grown.

TABLE 3

Percentage root length colonized by VAM fungi in the field[1]

Farming system	1990		1991	
	Maize	Soybeans	Maize	Soybeans
Low-input with animals	45 a	39 b	49 a	–[2]
Low-input cash grain	35 b	67 a	33 c	37 a
Conventional	35 b	9 c	43 b	37 a

[1]Soybeans were sampled 16 July 1990 and 26 June 1991; five plants per plot, four plots per farming system. Maize were sampled 28 June (LIA and CONV) and 16 July (LICG) 1990 and 18 June (LIA and CONV) and 26 June (LICG) 1991; ten plants per plot, four plots per farming system. The difference in sampling dates was due to differences in planting dates. Numbers in the same column followed by the same letter are not significantly different ($\alpha=0.05$, Tukey's Method of Multiple Comparisons).
[2]Soybeans were not grown in 1991 in the plots sampled.

TABLE 4

Percentage root length colonized by VAM fungi in a greenhouse assay using 1:1 (v/v) mixture of field soil and vermiculite[1]

Farming system	Colonization of host plant		
	Maize (1990)[2]	Bahiagrass (1991)[3]	
	Soil planted to maize in 1989	Soil planted to maize in 1990	Soil planted to soybeans in 1990
Low-input with animals	31 a	10 b	9 a
Low-input cash grain	31 a	14 a	10 a
Conventional	13 b	2 c	1 b

[1]Numbers in the same column followed by the same letter are not significantly different ($\alpha=0.05$, Tukey's Method of Multiple Comparisons).
[2]Mean of four observations, after 4 weeks of growth. Soil was collected from the field on 20 April, 1990.
[3]Mean of seven observations, after 3 weeks of growth. Soil was collected on 15 May 1991.

rhizal colonization than those in soil from CONV plots (Table 4). There was no significant difference in root or shoot weights, but plants in soil from the LISA systems had lower root:shoot ratios than those in soil mix from the CONV plots (0.76 vs. 1.22, $\alpha=0.05$).

Bahiagrass seedlings were grown for 3 weeks in the greenhouse in cones with 1:1 (v/v) vermiculite:soil collected 15 May and 23 October, 1991. Soils planted to both maize and soybeans were assayed, so bahiagrass, a general host commonly utilized in pot cultures, was grown. Entire root systems were assayed for mycorrhizae and showed substantially greater colonization in soil from LIA and LICG than CONV for both crops (Table 4).

DISCUSSION

Farming systems affect VAM fungi in the soil. Prior to the experiment, the field had a history of use for a maize–wheat rotation. Variability in distribution of VAM fungi at that time was not the result of crop factors because the crop was the same across the field. Factors which affected the distribution of VAM fungi were soil type, drainage, etc. which still impacted upon the fungi in this experiment. Because these factors were accounted for in the present experimental design, the differences in VAM fungi across plots must be the result of farming system. It is not known when the observed differences manifested themselves during the 10 year period of the experiment.

Qualitative differences between the LISA and CONV farming systems could be the result of several factors. Diversity of VAM fungi has been shown to be proportional to vegetative diversity (Rabatin and Stinner, 1989). The CONV rotation consisted of only two crops, whereas the LISA systems had at least five different crop species. In addition, chemical inputs to the CONV system may have altered the species dominance among the VAM fungus population (Abbott and Robson, 1991).

Quantitative differences between the LISA and CONV farming systems may have arisen from the greater availability of host plants in the LIA and LICG systems (Fig. 2). Warm, wet weather after harvest in autumn or before sowing in the spring can lead to a reduction in vigor of VAM fungi in conventionally farmed soil. This also may have had an effect upon the inoculum potential of the soil (Table 4).

Other researchers have found little correlation between VAM fungus spore populations and colonization of roots in the field (Kruckelmann, 1975; Abbott and Robson, 1982). This has been attributed to differential rates of colonization by different fungi. Also, undisturbed field soil may contain propagules other than spores, such as viable hyphae and colonized roots. This may explain why results of field and greenhouse studies of soil inoculum potential differed. Abbott and Robson (1991) recommend the use of a greenhouse bioassay, as used here. They found a good relationship between the colonization of 3-week-old-plants in the greenhouse and the colonization of field grown plants near the end of the growing season. Soil under the LISA systems studied here had greater capacity to produce VAM fungus colonization. In other work, a change from conventional, high input agriculture to a LISA system resulted in a 3–4 fold increase in colonization of roots of potato and wheat in the Netherlands (Limonard and Ruissen, 1989).

Gigaspora gigantea has large, brightly colored spores which are readily identified. Thus, it was fortuitous that this species was affected by farming system treatment. The populations of *Gigaspora gigantea* showed a marked rotation effect, being greater following maize than soybeans. This is consistent with greenhouse data which have shown maize to be a better host than soybeans for the production of VAM fungus spores (Struble and Skipper, 1985).

Mosse (1986) stated that "It is as normal for the roots of plants to be mycorrhizal as it is for leaves to photosynthesize. Any agricultural operation that disturbs the natural ecosystem will have repercussions on the mycorrhizal system". It is evident from data presented here that there is a larger population of VAM fungi in soil under low-input than under conventional agriculture. More research is needed on the contribution to crop growth and yield by these fungi relative to that of creative nitrogen/soil cover management found in LISA.

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